Cutting and pasting polyenes

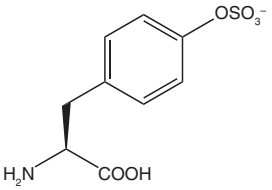
Spectinabilin, a polyketide found in several *Streptomyces* spp. strains, is a polyene metabolite constructed from acetate, propionate and nitrobenzoic acid equivalents. Recent studies have suggested that spectinabilin may itself serve as a biosynthetic precursor to two metabolites, SNF4435C and SNF4435D, which are isomers that contain a cyclobutane core element. Müller *et al.* now provide evidence that a series of nonenzymatic electrocyclization reactions may provide a biosynthetic avenue to SNF4435 compounds and related polyketides. Metabolic profiling of *Streptomyces orinoci* showed that SNF4435 isomers are produced from spectinabilin only in the presence of light. The authors observed that photochemical isomerization of the double bonds in spectinabilin can initiate two sequential electrocyclic reactions leading to SNF4435C and SNF4435D. The mechanistic proposal also suggested an alternative biosynthetic route to orinocin, a polyketide that is structurally related to spectinabilin but lacks a central triene unit. The authors hypothesized that orinocin could be derived from spectinabilin by 'polyene splicing': SNF4435 isomers, formed from spectinabilin by the tandem electrocyclic reactions, could undergo a retro[2+2]cycloaddition that would lead to orinocin and produce mesitylene as an aromatic byproduct. Indeed, direct irradiation of SNF4435C did produce orinocin and mesitylene as a 'splicing' product. The polyene splicing mechanism offers an alternative mechanistic framework for understanding polyketide structural tailoring. (*Angew. Chem. Int. Ed.*, published online 25 October 2006, doi:10.1002/anie.200602840) *TLS*

The ABCs of AmPs

Peptides with activity against microbial invaders are generated by the innate immune system of eukaryotes. Such antimicrobial peptides (AmPs) are approximately 20 amino acids in length and have a net positive charge that facilitates their binding to and ultimate destruction of the outer leaflet of the microbial membrane. Because of their small size, AmPs seem to rely less on tertiary and quaternary structures and more on their peptide sequence for function. Therefore, Loose *et al.* hypothesized that they could generate non-natural AmPs based entirely on the language revealed by these sequences. With no assumptions based on structure-activity information and no knowledge of AmP interactions with membranes, the authors modeled sequences from a database of 526 well-characterized AmP sequences. They treated the AmP sequences as a language in which the 'grammars' are decamer rules that reflect the common arrangements of amino acids obtained from the database sequences. From the ~700 grammars the authors identified, they generated *in silico* AmP-length peptides that had limited homology to known proteins by random arrangements of two grammars. They then synthesized a subset of these and found that 45% of the new AmPs do indeed inhibit growth of at least one of two bacterial targets, *Bacillus cereus* or *Escherichia coli*. Two of these peptides, as well as 18 variants of one of them, had minimum inhibitory concentrations comparable to those of positive antibiotic controls and were active against a drug-resistant *Staphylococcus aureus* strain and against *Bacillus anthracis*, the causative agent of anthrax. This approach demonstrates the modular nature of AmPs and provides a strategy for generating antibiotics that are active against pathogens that tend to become resistant to traditional antibiotics. (*Nature* **443**, 867–869, 2006) *MB*

Research Highlights written by Mirella Bucci, Catherine Goodman and Terry L. Sheppard

Incorporating sulfotyrosine



Hirudin is a protein secreted by the leech *Hirudo medicinalis*; it is widely used as an anticoagulant owing to its tight binding and competitive inhibition of thrombin. Hirudin sulfation on a single tyrosine residue is responsible for much of its affinity for thrombin. Therefore, it is desirable to optimize the conditions for incorporation of sulfotyrosine for production of hirudin. Liu and Schultz have now used a general nonsense suppression strategy to allow for incorporation of sulfotyrosine into hirudin and other secreted and membrane-bound proteins. This suppression strategy is based on an orthogonal tRNA/aminoacyl-tRNA synthetase (aaRS) pair that allows incorporation of unnatural amino acids in response to the amber nonsense codon. To find an aaRS that incorporates sulfotyrosine, the authors generated a library of active site mutants in the coding sequence of a gene encoding a tyrosyl-tRNA synthetase that charges an engineered suppressor tRNA at the nonsense codon. One of these mutants, STyrRS, showed specific and efficient sulfotyrosine incorporation when expressed in *E. coli* along with the suppressor tRNA and a plasmid encoding a generic amber-containing protein. They then generated sulfohirudin and confirmed that it is a stronger inhibitor of thrombin activity than desulfohirudin. Applying STyrRS to uniquely incorporate sulfotyrosine will be useful for structure-function studies of the coagulation cascade as well as the various pathways where sulfotyrosine is incorporated. (*Nat. Biotechnol.*, published online 29 October 2006, doi:10.1038/nbt1254). *MB*

Lysine steals a proton

Vitamin K–dependent carboxylase catalyzes the carboxylation of glutamic acid residues to create calcium-binding modules necessary for hemostasis and other biological functions. Initial work suggested that the transformation happens in two steps, in which the protein first deprotonates vitamin K with a weak base, followed by reaction of vitamin K with oxygen to form a base powerful enough to deprotonate the γ-carbon of glutamic acid in a mechanism called the base-amplification model. Rishavy *et al.* have now identified the active site base in a search aided by evolution. Initial work ruled out a cysteine as the base but left the authors with a plethora of remaining possible candidates. The discovery of a modified carboxylase in a bacterial pathogen that can perform the first reaction to deprotonate vitamin K (but not the second) focused the search onto only four reactive residues in common between the bacterial and eukaryotic homologues. Mutation of three identified histidines had a minimal effect, whereas substitution of Lys218 resulted in complete loss of enzyme activity. The reactivity of the K218A mutant could be restored by exogenous amines in a concentration-dependent manner, providing strong support that changes in activity are not due to secondary effects. The successful identification of Lys218 as the active site residue is an important first step in unraveling the secrets of this enzyme. (*Biochemistry*, published online 18 October 2006, doi:10.1021/bi0609523) *CG*